

Patterns of morphospecies richness, diversity, and community similarity across a hair density gradient in the human skin microbiome

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Introduction

“Everything is everywhere, but the environment selects,” proposed Baas Becking and Beijerinck (De Wit & Bouveir, 2006). This pithy statement encapsulates the idea that niche selection is the main deterministic process in determining species presence or absence from an area (De Wit & Bouveir, 2006). However, dispersal ability and stochastic processes are also known to affect species presence. While both processes are recognized today as influencing species distribution and community assembly, it still remains an open area of investigation as to which process is most influential and in which conditions (De Wit & Bouveir, 2006). Some studies find that habitat heterogeneity predicts beta diversity more than stochastic processes and distance, while others find that geographic distance explains most turnover between communities, suggesting dispersal limitation may be the main driver of community assembly (Astorga et al., 2014; Bin et al., 2010; McClain & Barry, 2010; Wen et al., 2016). Some suggest that this may be because dispersal limitation affects community composition at a larger spatial scale, while habitat heterogeneity determines species presence at a more local scale (Freestone & Inouye, 2006; Martiny et al., 2006). However, our understanding of these drivers of diversity is biased due to a disproportionate focus on vertebrate research (Tews et al., 2004).

Thus, an exciting new area of research in ecology is applying these biogeographical concepts to predict the distribution and community assembly of microorganisms. For example, one of the key rules of biogeography, the taxa-area relationship, has also been shown to hold true for bacteria (Horner-Devine et al., 2004). This rule states that the larger the area, the more species can be supported by that area, and it can be explained by including more species with limited dispersal ability or by gaining more species of different niches with increasing spatial scale (Horner-Devine et al., 2004). The latter explanation is favored for bacteria, which are generally assumed to be excellent dispersalists (Martiny et al., 2006). Therefore, it is not surprising that geographic distance reduced bacterial community similarity primarily through increased environmental heterogeneity across English salt marshes rather than via geographic distance alone (Horner-Devine et al., 2004).

Experimental manipulations have also been able to address this question by using the human skin microbiome. It has been well-established that cutaneous sites vary in microbial composition and diversity (Costello et al., 2009; Kong & Segre, 2012). This variation could either be explained by differences in the microbes' ability to disperse to that skin region, including historic exposure of the skin, or due to environmental heterogeneity, such as differences in skin pH, temperature, oxygen levels, and nutrient availability (Grice & Segre, 2011). However, studies have shown that when the forehead is disinfected and inoculated with foreign bacteria, the introduced community becomes more similar to its former, native community over time, further supporting the idea that environmental selection rather than dispersal limitation is the main driver of differences in species composition across regions (Costello et al., 2009). Additionally, when the same body sites' microbial communities were sampled from different individuals, they were shown to be more similar in composition than different sites on the same individual, suggesting that habitat heterogeneity is the main cause of variation in bacterial species presence (Grice et al., 2009).

In particular, one of the major causes of variation in skin surface environment is the distribution of sebaceous glands (Grice & Segre, 2011). These glands are known for their association with hair follicles and secrete sebum. Sebum is an oily substance that coats the hair and creates an oxygen-deprived environment, such that these environments favor the growth of anaerobic and lipophilic bacteria more than skin sites

with few sebaceous glands (Grice & Segre, 2011). Therefore, because sebum secretion facilitates the growth of microhabitats, in this study we predicted that across human individuals, a site with a greater number of hair follicles would support the highest microbial morphospecies richness relative to sites with lower densities of hair follicles. We also predicted that morphospecies diversity would be highest in the region of highest habitat heterogeneity (highest hair follicle density). Thus, between individuals sampled, those with higher hair follicle densities would harbor microbial communities of higher morphospecies richness and diversity. Finally, we expect that the similarity between communities will differ based on differences in hair follicle density with the site containing the highest hair follicle density and the site with the fewest hair follicles being the least similar.

Methods

We randomly selected and sampled 20 male, brown-haired college students in the Donnelley and Lee Library of Lake Forest College in Lake Forest, IL. We controlled the sex and age of each participant because both are known to affect human microbiome composition (Kong & Segre, 2012). Each participant was swabbed with a sterile Q-tip at three cutaneous locations along a hair density gradient: 1) just behind the ear at the hairline (highest hair density) 2) the outer shin approximately 10 cm from the ankle (medium hair density), and 3) the inner forearm three cm below the elbow (lowest hair density). Each location and individual was swabbed using medium pressure for 3 seconds back and forth across a line approximately 3 cm long. Each participant was also asked how many hours ago they had last showered and we recorded their answer. We recorded each sample with a unique identifier for the participant and the location site. Arm and shin sites were both photographed using a smartphone camera with a ruler for reference (Fig.1). The number of hair follicles within a 3 cm by 3 cm square for both arm and leg sites were then counted. Due to poor depth resolution of the images, hair strands that were within the square were assumed to originate from follicles in that area.

After each sample, the Q-tip was submerged and swirled in PBS. To plate the samples, they were first diluted with PBS using a 1:20 dilution. Each of the diluted samples was mixed and then 50 ul of the solution was pipetted onto an agar plate. Five to seven glass beads were added and the plate was swirled vigorously for 10 seconds. These samples were plated no more than an hour after collection. The plates were then left in the incubator at 36°C for 72 hours to allow the microbes to grow. After 72 hours, the inoculated dishes were placed in a refrigerator to halt any further microbial growth. The dishes were then removed from the refrigerator and examined in order to determine morphospecies richness, abundance, and diversity. Morphospecies were determined to be relatively reliable proxies for taxonomic unit species for studying patterns of species richness and diversity (Derraik, et al., 2010). To categorize morphospecies, the form, margin, size, color, and elevation of each colony were recorded and 16 distinct morphospecies were identified (Table S1). Each morphospecies was then given a unique name.

The abundance (total number of colonies for each morphospecies) and richness (number of morphospecies) of each plate were recorded. The Jaccard similarity index, which measures the proportion of species shared between two sites relative to the total number of species of both sites, was calculated between each site for each individual (eg: between the shin and the forearm site) using the formula described in Muellenberg-Dombois & Ellenberg (1974). Then, an ANOVA was used to test whether the hairline and forearm sites were less similar within individuals than the similarity between the forearm and the shin or the similarity between the shin and the hairline. The morphospecies diversity of each sample was also calculated in Excel using the Shannon-Wiener diversity index, which incorporates both richness and abundance to measure the evenness of a community as described in Spellerberg and Fedor (2003). To determine whether morphospecies richness and/or diversity was predicted by sampling location site, we used ANCOVA tests using the number of hours since the participants had last showered; this was added to the model as a covariate to control for changes in microbial presence due to washing (Kong & Segre, 2012). We then used multiple linear regressions to test whether the hours since the last shower or the hair density on the arm or leg predicted the richness or diversity of morphospecies within those sites. All statistical tests were performed in R Version 3.3.1 and all figures were drawn in Microsoft Excel 2016.

Results

Richness

When tested with an ANCOVA, sampling location was not a significant factor in predicting morphospecies richness ($F_{2,54}=0.573$, $p=0.565$, Fig. 1). A linear regression of the number of hours since the last shower taken by the participant also failed to predict species richness at any site ($R^2=.03$, $p=0.088200$, Fig. 2). Linear regression of hair density for both the forearm (Fig. 3) and shin (Fig. 4) revealed that it did not predict species richness for either site ($R^2=0.061$, $p=0.1063$ and $R^2=0.024$, $p=0.381$).

Diversity

The ANCOVA did not reveal any significant difference in morphospecies diversity between sampling locations of different hair densities ($F_{2,56}=0.565$, $p=0.572$, Fig. 5) and the number of hours since showering also had no effect ($R^2=0.026$, $p=0.116$, Fig. 6). The multiple linear regression models for arm hair density and for shin hair density (both with hours since shower as an additional factor) were both nonsignificant (arm site: $R^2=0.1414$, $p=0.106$, Fig. 7; shin site: $R^2=0.002$, $p=0.381$, Fig. 8). Identity and frequency of occurrence

A bar chart was made comparing morphospecies identity with frequency, or number of times the morphospecies was observed for each site (Fig. 9). For all three sampling sites, Round White Ones were the most frequently observed, with 12 observations in the head and shin locations and 8 observations in forearm samples. Several morphospecies were found in only one or two sites. In general, the frequency of observation from the forearm was similar to the patterns of frequency seen from the head location, but the shin sampling site seemed to be more unique. Similarity

The ANOVA between the Jaccard indices, as calculated between each pair of sites within each individual, revealed no significant difference in the similarity between the hairline and forearm sampling sites, which were predicted to be the least similar, and the similarity between the hairline and shin site as well as the similarity between the forearm and shin sites showed no significant difference either ($F_{2,57}=0.566$, $p=0.571$, Fig. 10). Therefore, no pair of sites sampled was more or less similar in morphospecies composition than any other pair of sites from the same individual.

Discussion

Our prediction that morphospecies richness and diversity would vary between sampling site, with the hairline site (highest hair density) supporting the highest number of species, was not supported by our results (Figs. 1, 5). Therefore, hair density variation between different sites on the body does not appear to be a significant factor in determining microbial richness nor diversity at the morphospecies level. Further, morphospecies richness and diversity within the forearm and shin sites also did not increase with an increase in hair follicles (Figs. 3-4, 7-8). Taken together, these results suggest hair follicle density does not have any effect on morphospecies richness or diversity. Our results also do not support our prediction that the extremes of the hair density gradient (forearm and hairline sites) would be significantly less similar in morphospecies composition, measured using the Jaccard similarity index, as compared to the other two pairs' similarity indices. Thus, hair density, our proxy for overall habitat heterogeneity in this study, did not significantly influence microbial community composition at the morphospecies level (Fig. 10).

From the lack of a significant difference in morphospecies richness, diversity, and community similarity, we can also conclude that dispersal limitation does not seem to be a significant factor in differentiating the three sample sites' microbial composition. This conclusion is also supported by the observation that only three morphospecies were unique to one of the sampling sites and 11 of the 16 morphospecies characterized were present at all three sites across the hair density gradient (Fig. 9). Instead, our results suggest that neither dispersal limitation across the body (from the hairline to the shin) nor habitat heterogeneity (as facilitated by differences in hair density, which can cause environmental variation via sebum secretion) limit microbial morphospecies presence. It thus follows that our sampling sites were either too similar in environmental conditions or the sites were too close to limit dispersal; we cannot conclude whether one mechanism is more influential on human skin microbiota than the other.

These results are inconsistent with previous studies that have found a significant difference in microbial diversity across different cutaneous sites (Costello et al., 2009; Kong & Segre, 2012). However, these studies used the phylogeny metric UniFrac, rather than morphospecies, to determine community similarity. Therefore, it is possible that these studies were able to determine which sites were more clustered in relatedness due to niche selection at those sites by using phylogeny, while our assay was not sensitive enough to capture these differences. It is also possible that our study was simply underpowered since we only recruited 20 participants.

We also did not find the number of hours since the participants last showered to have any effect on morphospecies richness or diversity across sampling sites (Figs. 2, 6). This result is consistent with previous studies that found while microbial community composition is affected by washing, the overall diversity is not, either because microbes quickly re-colonize the skin or because washing is not sufficient to remove most microbes (Fierer et al., 2008). Future research could track the successional stages of colonization to determine how quickly this process occurs.

Additionally, previous studies have identified significant interpersonal differences in skin microbial composition (Costello et al., 2009; Oh, et al., 2016). A common assumption in many studies is that dispersal changes the biota at the large spatial scale, while niche selection operates on a more local scale, as has been demonstrated for some organisms (Freestone & Inouye, 2006). Future research should aim to resolve this dichotomy and unify these processes, as they do not operate independently (Schmidt et al., 2000). The microbiome is a convenient analogue for a large land mass with diverse biota with which to test theory because it is easy to cause mass extinctions and introduce new biota. However, few studies have taken advantage of this. These interpersonal differences in microbiomes could be analogous to differences between large land masses. By approaching the microbiome from a metacommunity perspective, future research could better delineate how differences between species compositions emerge.

While there are many possible experimental design explanations for our failure to find any effect of hair density on microbial morphospecies richness, diversity, and similarity between communities, it is worth interrogating the assumption that increasing habitat heterogeneity always increases diversity. While it is an intuitive assumption that with an increase in the number of niches, species richness within that local area would also increase, this is not always the case. For example, habitat heterogeneity has been shown to reduce species richness if the region is isolated from dispersal because this increase in habitat heterogeneity reduces the amount of area available for each habitat, resulting in a higher likelihood for extinction (Kadmon & Allouche, 2007). However, this exception cannot explain our failure to find any relationship between habitat heterogeneity (as inferred from hair density). In particular, we found that only three morphospecies were restricted to one body site, suggesting there was no isolation of biota to result in a loss of area with increased habitat heterogeneity (Fig. 9). Instead, this concept could also be tested by assessing the species richness of different individuals' skin microbiomes with variation in hair density as a proxy for habitat heterogeneity and variation in exposures to microbes, which would be analogous to isolation. Since most publications on this idea are based on models instead of empirical studies due to the difficulty of manipulating these variables at a larger scale, it is exciting that this idea could be tested more easily using the human microbiome (Kadmon & Allouche, 2007).

Further, the effect of habitat heterogeneity on species richness has also been shown to be mediated by resource availability (Yang et al., 2015). Modeling has also revealed that environments with either a low amount of resources or a high amount of resources support more species with increasing heterogeneity of the environment, whereas intermediate-level resource environments have a nonlinear relationship with increasing habitat heterogeneity, first increasing in richness and then decreasing (Yang et al., 2015). Future research could experimentally manipulate the skin conditions to vary the amount of resources available for the human skin microbiota to test these proposed relationships in vivo and to better ascertain the mechanisms (competition, stress-tolerance) that underlie these relationships.

Moreover, it is also possible that perhaps the most common morphospecies, categorized as the 'Round White Ones' (RWO), are dominant competitors across the cutaneous environment, resulting in sim-

ilar patterns of coexisting morphospecies richness and diversity across the hair density gradient (Bruno et al., 2003). However, because of the few samples in our study, we did not have sufficient data to statistically test whether presence of RWOs predicted morphospecies richness or diversity. It is important to note, however, that competition and species interactions, in addition to habitat heterogeneity, can also affect species presence and future studies could experimentally monitor the competition between microbe species of the skin (Hibbing et al., 2010).

While this study was limited by the fact that we did not identify microbes to taxonomic units, calculate niche breadth, or determine the strength of competition between the microbes, the results of this study illustrate that more research is needed to determine the effects of dispersal limitation and habitat heterogeneity on skin microorganism communities. Applying biogeographical concepts to microbiomes can lead to both a greater understanding of microbial community composition and diversity, as well as allowing one to test these concepts that are difficult to describe and manipulate at a macroorganismal level.

Note: Eukaryon is published by students at Lake Forest College, who are solely responsible for its content. The views expressed in Eukaryon do not necessarily reflect those of the College.

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Figure descriptions

Figure 1: Average morphospecies richness for each sampling location, where head is the highest hair density and forearm is the lowest hair density. Error bars are standard error for each mean.

Figure 2: Scatterplot of morphospecies richness vs. hours since last shower for each location.

Figure 3: Scatterplot of morphospecies richness of forearm vs forearm hair density. Regression line is not shown because it was nonsignificant.

Figure 4: Scatterplot of morphospecies richness of shin vs shin hair density. Regression line is not shown because it was nonsignificant.

Figure 5: Average Shannon-Wiener Diversity Index of morphospecies for each location. Error bars are the standard error for each mean.

Figure 6: Scatterplot of morphospecies Shannon-Wiener Diversity Index vs. hours since last shower. Regression line is not shown because it was nonsignificant.

Figure 7: Scatterplot of forearm morphospecies Shannon-Wiener Diversity Index values vs. forearm hair density. Regression line is not shown because it was nonsignificant.

Figure 8: Scatterplot of shin morphospecies Shannon-Wiener Diversity Index values vs. shin hair density. Regression line is not shown because it was nonsignificant.

Figure 9: Morphospecies incidence frequency (the number of individuals that morphospecies was found on that sampling site) for all morphospecies, as described in Table S1.

Figure 10: Mean Jaccard similarity index between each site pair comparison, between the head and forearm, the head and shin, and the forearm and shin. Standard error bars are standard error for each mean.

Table S1: Morphospecies identifier names, with distinguishing characteristics size, color, margin, and elevation.

Figure 1:

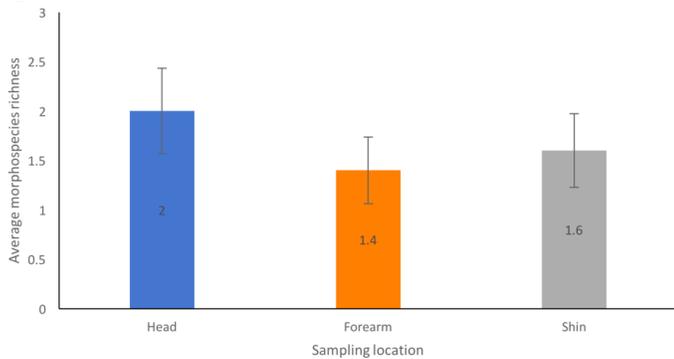


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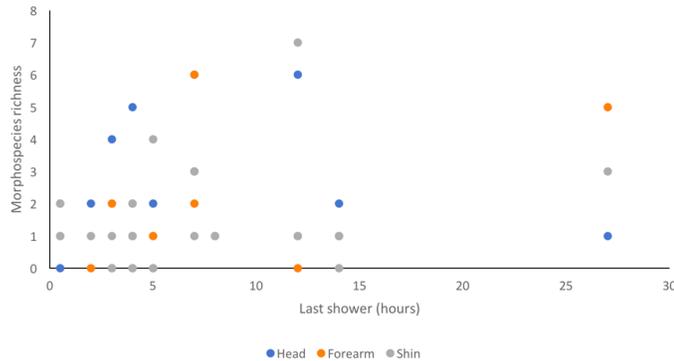


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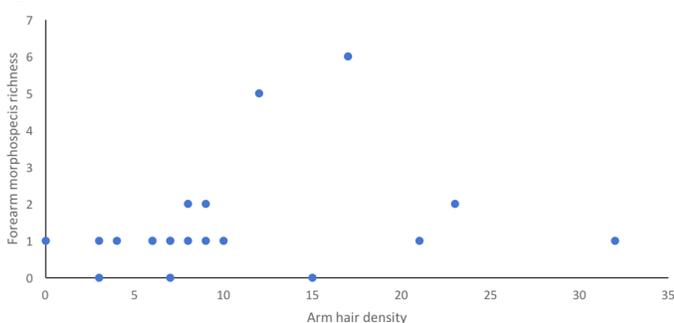


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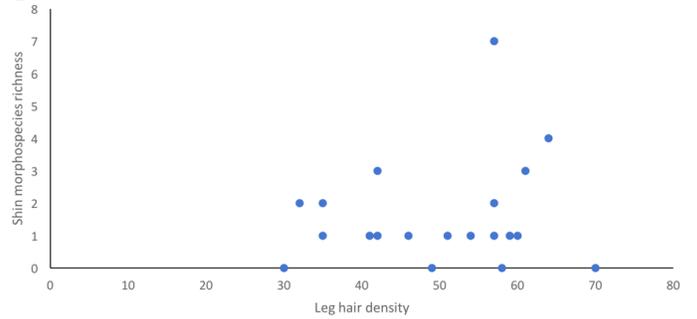


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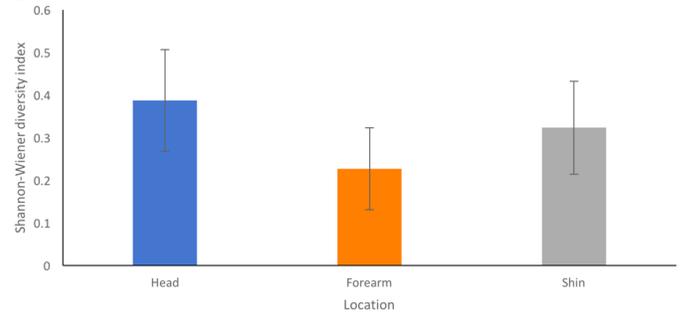


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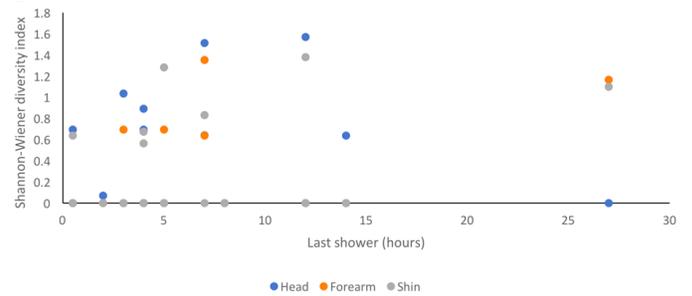


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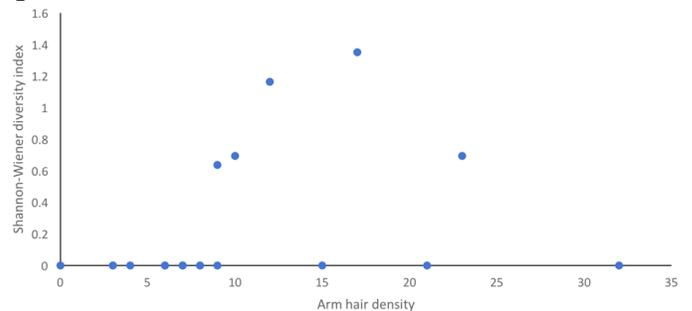


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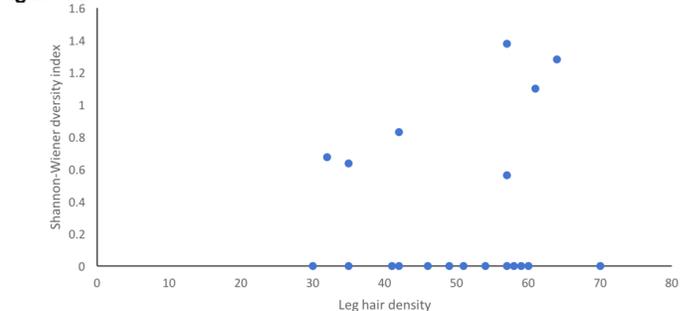


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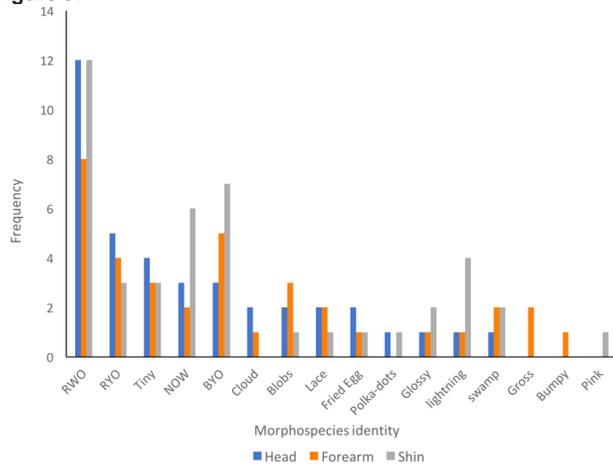


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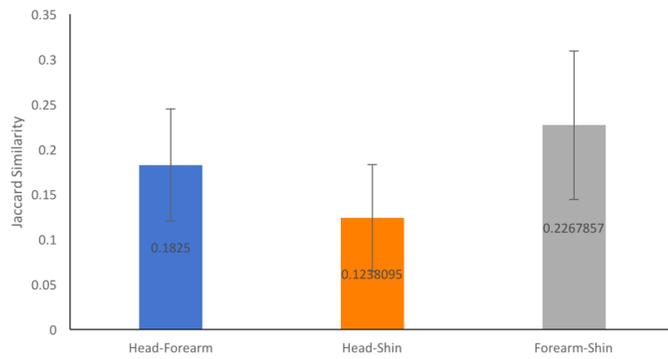


Table S1:

Name	size	color	margin	elevation
Cloud	medium	off white	undulate	flat
Tiny	tiny	white	entire	flat
RWO	small	white	entire	raised
polkadots	small	white	entire	flat
gross	large	white edges brown center	entire	crater form
RYO	small	yellow	entire	flat
BYO	small	yellow	entire	raised
lightning	huge	white	lobate	flat
blob	huge	white	undulate	flat
lace	small	white	undulate	irregular
NWO	small	white	entire	raised
swamp	large	brown-ish white	undulate	flat
fried egg	small	white with a yellow center	undulate	flat
glossy	tiny	yellow	entire	raised
bumpy	small	yellow	undulate	irregular
pink	small	white with pink center	entire	convex